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AN EXAMINATION OF MERCURY CONCENTRATIONS IN EGGSHELLS OF THE COMMON SNAPPING TURTLE (*CHELYDRA SERPENTINA*) IN NOVA SCOTIA, CANADA

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ABSTRACT

Mercury (Hg) is a potentially toxic metal that has bioaccumulating and biomagnifying properties. In egg laying animals, it can be transferred from an adult female to offspring. However, in turtles, the inter-and-intra-nest variation of Hg concentrations remains unknown. We investigated the concentration of Hg in preyed-upon Common Snapping Turtles (*Chelydra serpentina)* eggshells. The variability in Hg contamination between and within each nest was assessed. In June 2021, 368 eggshells left behind by predators were sampled from 14 nests, from three different sites in southwest Nova Scotia. Ten eggshells were randomly selected from each nest for analysis. We found no correlation between estimated number of eggs in a nest and average nest Hg concentration. Significant inter-nest variation (Hg ranging from 12.0 ± 3.85 to 172.3 ± 43.9 µg/kg) and intra-nest variability may indicate maternal transfer. The collection of the shells of freshly preyed- upon eggs is a useful non-destructive sampling technique to maintain sustainable turtle populations. Our results demonstrate the need for further investigation into the impact of Hg on temperate, freshwater turtle reproduction.

Keywords***:*** mercury concentration, maternal transfer, common snapping turtle, eggshells

INTRODUCTION

Mercury (Hg) is a heavy metal contaminant that lacks any biological function but has widespread prevalence and is toxic to humans and wildlife (Scheuhammer *et al.* 2007). Aquatic ecosystems are especially sensitive to the deleterious effects of elevated exposure

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to Hg (Singh *et al.* 2011). The effects include decreased reproductive success, impaired hormone production, alteration of offspring phenotype, behavioural changes and even death (Scheuhammer *et al.* 2007, Singh *et al.* 2011). Due to its bioaccumulating and biomagnifying properties, mercury can be maternally transferred from female to offspring in eggs in many oviparous taxa (Mason *et al.* 1996). For example, female American toads (*Bufo americanus*) collected from a Hg-contaminated site transferred approximately 5% of their Hg body concentration to their egg clutches which resulted in reduced hatchling success (Bergeron *et al.* 2010). Maternally transferred Hg from eggs in hatched American toad juveniles has also been associated with reduced body size and impaired larval swimming performance (Bergeron *et al.* 2011a). In tree swallows (*Tachycineta bicolor*), Hg exposure was correlated with both decreased female reproduction and fledging success (Bergeron *et al.* 2011b).

Little is known about the reproductive effects of Hg in turtles. Turtles are commonly used to monitor Hg exposure in contaminated areas because of their ecological and life-history characteristics (Green *et al.* 2010, Hopkins *et al.* 2013a, de Solla *et al.* 2004). The Common Snapping Turtle (*Chelydra serpentina*) has potential to be a useful species for biological monitoring of environmental Hg contamination as this turtle is non-migratory with a small home range (Obbard and Brooks 1981), and is therefore suitable to monitor local contamination (Golet and Haines 2001). Common Snapping Turtles are long-lived (over 100 years in the wild, COSEWIC 2008) and apex predators, both of which make them highly susceptible to Hg bioaccumulation and biomagnification (Hopkins *et al.* 2013a).

The Common Snapping Turtle is found from Nova Scotia to Saskatchewan, and throughout the United States to the Gulf of Mexico (Conant 1975). The species is the largest freshwater turtle found in Canada and can weigh up to 30 kg (Conant 1975) with a maximum carapace length of 49.4 cm (Ernst and Lovich 2009). The Common Snapping Turtle is an opportunistic omnivore which consumes about one-third of its diet as fish, one-third as vegetation, and one-third as other organisms including amphibians, reptiles, birds and bird eggs, crustacea, and other invertebrates (Alexander 1943). In Canada, the species has been listed as being of Special Concern on Schedule 1 of the *Species at Risk Act* since 2011 (SC, c. 29, 2002) and as a vulnerable species under the Nova Scotia *Endangered Species Act* since 2013 (SNS, c. 11, s. 1, 1998).

The Common Snapping Turtle, like most turtle species, has certain life-history traits that limit its ability to adapt to high levels of disturbance (Congdon *et al.* 1994, Gibbon *et al.* 2000). Throughout Canada, the Common Snapping Turtle is still widespread and relatively abundant. However, its late maturity, extended longevity, low recruitment and requirement for long, warm summers to successfully incubate its eggs, puts it at greater risk to anthropogenic threats (COSEWIC 2008). Common Snapping Turtles are highly susceptible to road mortality, especially on roads that run through or are adjacent to wetlands (Beaudry *et al.* 2008), and forecasting the effect of interventions on an endangered population requires an understanding of the spatial scales at which threat processes operate. Road mortality is among the greatest threats to semi-terrestrial freshwater turtles due to the group's life-history traits. Female Common Snapping Turtles nest for the first time between 17 and 19 years of age (Galbraith and Brooks 1989) with an average clutch size of 25-45 eggs (Ernst and Lovich 2009). The eggs are generally laid in nests which are dug in sand or gravel banks near the water, in locations with sparse vegetation. Females exhibit strong nesting site fidelity, returning to the same site in subsequent years (Loncke and Obbard 1977, Obbard and Brooks 1981). Populations experience a high rate of predation on eggs, with nest predation rates ranging from 59% to 94% (Congdon and Breitenbach 1987). Survivorship is low for hatchlings, with only a 6.4%-23.0% survival rate, in contrast survival is 93.0%-96.6% for mature adults (Heppell 1998). Current estimates of survival rates do not take into account Hg accumulation or maternal transfer. The federal management plan includes only minimal information on the risks of mercury to the species (Environment and Climate Change Canada 2016).

Given the vulnerability of freshwater turtle populations in North America, it is desirable to find ways of conducting ecotoxicology analyses on wild populations without sacrificing live turtles or still-unhatched whole eggs (Hopkins *et al.* 2013a). There are several non-lethal methods used to sample turtles, such as: whole eggs, claws/nails, blood, muscle biopsies, shell scute clippings and eggshells. Hopkins *et al.* (2013a) found that Hg concentrations in whole eggs were strongly and positively correlated with Hg levels in female muscle tissue. The authors also found that Hg concentrations negatively correlated with hatching success, as indicated by increased egg infertility and embryonic mortality. Avian and non-turtle reptile studies show that those taxa transfer Hg and other potentially toxic elements to their eggshells (Burger 1994). Hg concentrations have been found to be higher in the contents of whole eggs than in eggshells in warm-water slider turtles (*Trachemys scripta*) from South Carolina, but trace elements were still detectible in eggshells (Burger and Gibbons 1998). Additionally, Hg concentrations in eggshells of several marine turtle species including green (*Chelonia mydas*; Jian *et al.* 2021), leatherback (*Dermochelys coriacea*) and loggerhead (*Caretta caretta*) turtles, are correlated with Hg concentrations in local coral reef sediments (du Preez *et al.* 2018). Currently, the relationship between environmental Hg concentrations and eggshells is unknown for temperate freshwater turtles.

In turtle eggshells, the intra-clutch variation is minimal for organic contaminants (i.e., polychlorinated biphenyls, PCBs; Bishop *et al.* 1994) but the intra-clutch variation of Hg concentrations in eggshells remains unknown (Hopkins *et al*. 2013b). As a result, extrapolation from current contaminant literature for turtle eggshells is difficult because organochlorine contaminants have different physiochemical properties (such as being more lipid-soluble) to those of Hg (which binds to and accumulates in protein tissues). The maternally transferred Hg quantity may decrease as clutch size increases, as the total contaminant burden is divided among a greater number of eggs. This merits further investigation given the vulnerable status of Common Snapping Turtles in Nova Scotia. The present study assesses the variability of Hg in eggshells collected from a range of nests, in order to understand the maternal transfer of Hg between and within nests in relation to whether the nests are in contaminated sites (near natural sources of environmental Hg) in Nova Scotia. This study may be the first report on mercury bioaccumulation trends in freshwater turtles in this region.

METHODS

Sample Collection

The Common Snapping Turtle is protected by the Species at Risk Act (Canada) and the Endangered Species Act (Nova Scotia), and permission to collect eggshell remnants was granted by Nova Scotia Lands and Forestry, Wildlife Division (now called Nova Scotia Department of Natural Resources and Renewables), under a Scientific/Species at Risk Permit.

Common Snapping Turtle nests were sampled at three southwestern Nova Scotia locations in Lunenburg County: Hebb Lake, Milipsigate Lake and Hebb Mill Brook (Fig 1). Sampling was conducted in partnership with Coastal Action, an environmental NGO based on the south shore of Nova Scotia. Nest monitoring and sampling were conducted as part of their ongoing Common Snapping Turtle monitoring program. All nests were monitored daily during June 2021, and all predation events which resulted in loss of eggs from each nest were noted. Approximately 368 eggshell fragments were

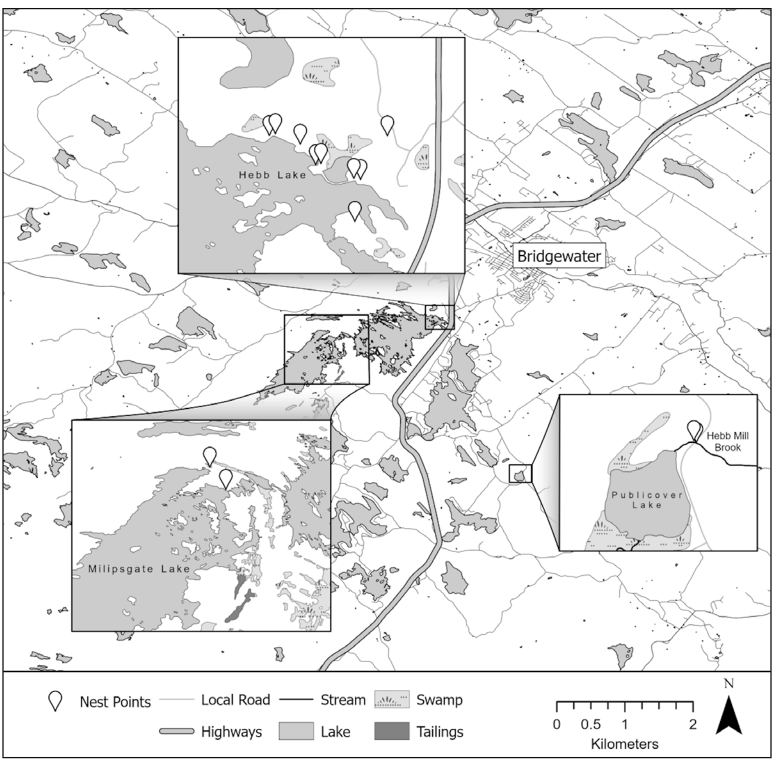

Fig 1 Map of the three sampled nesting sites located in southwest Nova Scotia: Hebb Lake, Milipsigate Lake and Hebb Mill Brook. All sites are within 10 km of each other. Map includes historical gold mining in the Leipsigate area, which includes the Milipsigate Lake nesting site in the current study.

collected within or in close proximity (<1 m) to nests, with collections taking place within 24 hours of the predation event. For each nest, all preyed upon eggshells were collected using latex gloves, wrapped completely in aluminum foil, individually sealed, and labelled with a nest identification number. All samples were stored in a portable cold storage chest until transferred to the laboratory freezer at Saint Mary's University until analysis.

Sample Preparation

Before analysis, eggshells were cleaned with a soft toothbrush and type-3 reverse osmosis (RO) water to remove all gravel, soil, and other external elements. Cleaned eggshell samples were air-dried for 48 h. The dried eggshells were then homogenized into fine powder using 10-vial adaptors for a Retsch MM400 mixer mill, with two 5-mm stainless steel surgical grade balls per micro-centrifuge tube (30.0 hertz/s for 2.5 minutes).

Instrumental Method and Quality Control

Total Hg (THg, µg/kg) of all samples (average sample weight 0.07 mg each) was analyzed using a Milestone Direct Mercury Analyser-80.3 © (DMA) at Saint Mary's University. Ten eggshells were selected at random from each nest to be analyzed, except for nest # MIL004, where only nine eggshells in total were collected. At the start of each analysis, certified reference materials (CRM) DORM-4 (dogfish muscle tissue from the National Research Council of Canada; certified Hg concentration: 0.412 ± 0.036 mg/kg), and DOLT-5 (dogfish liver from the National Research Council of Canada; certified Hg concentration: 0.44 ± 0.018 mg/kg) were used to validate the method and equipment. Additionally, a commercially available gardening soil supplement, GAIA Green Oyster Shell Flour (~36% calcium, gardening soil supplement), was used as a standard reference material approximating eggshell composition. Measured values (n=6) for DORM-4 were 0.392 ± 0.015 mg/kg; for DOLT-5 were 0.364 ± 0.009 mg/kg; and for GAIA were 0.002 ± 0.0005 mg/kg. Blanks were also included at the start of each analysis, and after every 10 samples. Six replicates of approximately 0.06 mg of eggshell samples were used as quality control to ensure the Relative Standard Deviation (RSD) was below 8% between measurements.

Statistical Analysis

Data were analyzed using the software Jamovi and RStudio, v.1.4.1 (*R development Core Team*). THg concentrations for each nest were plotted by site. The comparison of THg concentration between nests across all sites was assessed by ANCOVA. The variability of Hg concentrations at each site was assessed using Levene's test: homogeneity of variance. Furthermore, the relationship between approximate number of eggs in a nest and Hg concentration was determined using a correlation matrix. Significance for statistical analyses was always set at $p < 0.05$ unless otherwise noted.

RESULTS

Hg concentrations in eggshells were found to be the highest in Hebb Mill Brook nests, with averages ranging from 121.42 to 172.29 µg/kg (Fig 2). The ANCOVA also revealed a significant difference

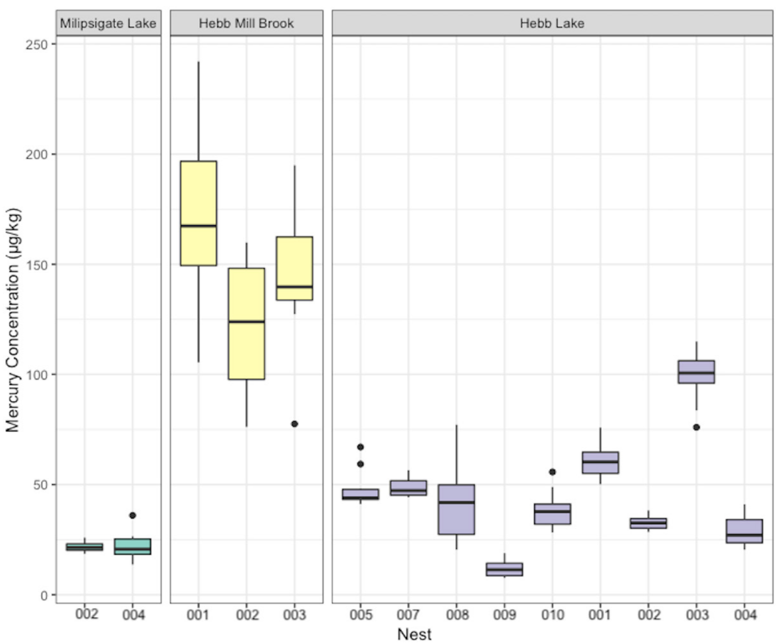

Fig 2 Mercury concentration (µg/kg) of eggshells from each sampled nest from all three study sites. Nest codes mentioned in text include ML00X (Milipsigate Lake), HB00X (Hebb Mill Brook) and HL00X (Hebb Lake).

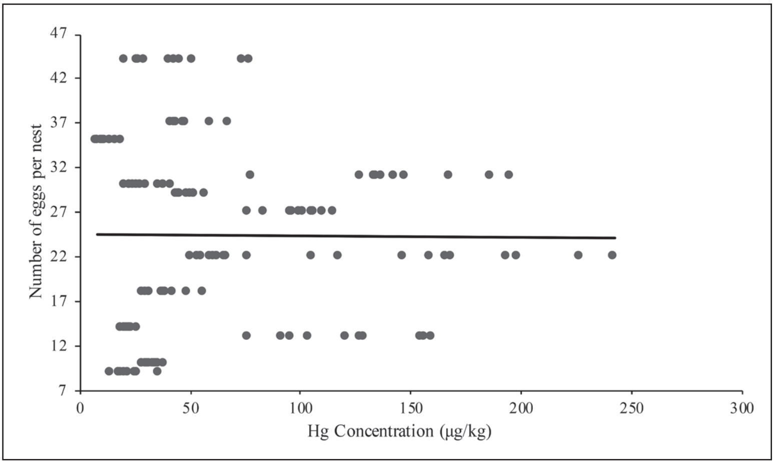

Fig 3 **The approximate number of eggshells in a nest and the Hg concentration was found to have no correlation (all sites combined), r(138) = -.029, p = .737.**

in eggshell Hg concentration among the 14 nests, $F(1, 13) = 73.6$, $p < 0.01$.

At Hebb Lake, Hg concentrations also varied significantly among nests ($p < 0.001$). For both Hebb Mill Brook and Milipsigate Lake, the averages of all nests at each site were considered to be equal ($p = 0.49$ and $p = 0.10$, respectively). However, these sites included three and two nests respectively, and do not hold high statistical power. Finally, the number of eggshells in a nest and the Hg concentration were not correlated, $r(138) = -.029$, $p = .737$ (Fig 3).

DISCUSSION

Average Hg concentrations of eggshells significantly differed among the three nesting sites, with highest levels of eggshell Hg at Hebb Mill Brook. In addition, we found a significant variation in average eggshell Hg concentrations among nests at Hebb Lake, and that there was no correlation between the number of eggshells within a nest (clutch size) and Hg concentration. It is clear that Hg is offloaded unequally and differently at each nest.

Maternal transfer of Hg to eggs has already been documented in several reptile and avian species. It has been found that Hg concentration in the eggs is directly influenced by the female Hg burden, which depends on habitat and diet (Ackerman *et al.* 2017, Heinz *et al.* 2010). In New York state, Hg concentrations in scute and soft-tissue samples from Common Snapping Turtles were correlated with more acidic water chemistry and atmospheric Hg deposition (Turnquist *et al.* 2011) as well as proximity to urban centers (de Solla *et al.* 2004). This leads to several possibilities which we will briefly consider here, including age and body size (larger and older turtles having accumulated more Hg over time); clutch size (larger clutches of eggs resulting in greater loss of mercury from laying adult); environmental exposure; and different bioaccumulation trends of mercury from diet.

Body size and age are often used in contaminant studies as it is presumed that older and larger individuals may have accumulated more contaminants over their lifetime. However, previous studies have found no relationship between muscle Hg concentration and body size in turtles (Golet and Haines 2001, Benjamin *et al.* 2018). Organochlorine contaminants have different physiological properties to mercury, but it has been determined that body and clutch size measurements do not significantly correlate with PCB concentrations in whole eggs. In addition, larger, older turtles, or those that lay the highest clutch size, mass, do not produce eggs that are more contaminated with organochlorine contaminants (Bishop *et al.* 1994). A published literature review of marine and freshwater turtles indicates that Hg concentrations are not correlated with body size (Benjamin *et al.* 2018).

Environmental Hg exposure for adult turtles remains a possibility. One significant potential exposure originally was expected to be historical gold mining sites. The Milipsigate Lake nesting site (Fig 1) is situated on a water body adjacent to historical gold mine tailings (Leipsigate Gold Mining District) which was historically processed using Hg amalgamation methods between 1860 and 1945 (Wong *et al.* 1999). Hebb Mill Brook is also located near a known gold-ore deposit which may have been associated with Hg amalgamation (Department of Energy and Mines 2021). Hg from historical tailings can leach into the surrounding environment through soil, aquifers, and water bodies (Wong *et al.* 1999). As a result, we expected the eggshells at Milipsigate Lake to have the highest concentrations of Hg, but this trend was not observed.

The Hebb Mill Brook site had the highest observed concentrations of Hg, and while located near a potential gold occurrence (Department of Energy and Mines 2021), other potential sources should be considered. The Hebb Mill Brook is a smaller water body, with relatively more adjacent wetland area than the other two sites which are predominately lakes. Wetlands are associated with rapid methylation of Hg and the associated increase of Hg in nearby food webs (Benjamin *et al.* 2018). Additionally, the proximity to roadways, with associated dust, tire break-down products and transport of industrial contaminants, has been observed to influence Hg levels (Lu *et al.* 2009), and the nests at the Hebb Mill Brook site were located directly on the gravel shoulder of a road. A third, but weaker, possibility for the elevated Hg in Hebb Mill Brook turtle eggshells would be that the female travelled further from contaminated sites to this nesting location. Common Snapping Turtles have non-migratory small home ranges (Obbard and Brooks 1981) with reproductive females typically travelling 2-4 km to lay eggs. However some females have been observed travelling up to 16 km (Obbard and Brooks 1980). Although, it is unlikely that three reproductive females travelled long distances from a contaminated site to lay eggs at this site, it cannot be entirely discounted due to a paucity of telemetry and tracking data for turtles in Nova Scotia.

The variation in Hg concentrations between the three sites might be associated with differences in maternal diet. While the three nesting sites in the current study were within 10 km of each other, significant variation in maternal diet, and subsequent Hg bioaccumulation from food webs, is likely. There is very little information on dietary patterns and mercury transfer in the Common Snapping Turtles in Nova Scotia. Information about diet and placement among trophic levels could be confirmed using stable isotope ratios of nitrogen and carbon of muscle tissues. In loggerhead turtles, stable isotope ratios have been observed to correlate positively with body size, indicating a trend of increasing trophic level with age (Godley *et al.* 1998). As Hg concentration biomagnifies in higher trophic levels, using stable isotope analyses on reproductive female turtles and their dietary items would allow for researchers to use non-lethal sampling methods to determine differences in diet and subsequently if diet is playing a role in elevated Hg concentrations. Diet of the reproductive females is likely an important factor in mercury bioaccumulation and maternal transfer, but remains unknown in Nova Scotia. Dietary variation compounded with habitat differences with variable Hg concentrations could play a role in Hg bioaccumulation levels for the Common Snapping Turtle adults and eggs.

This is the first study to examine maternally transferred Hg concentrations in the eggshells of temperate, freshwater turtles in Nova Scotia. For future research on turtles, the following recommendations should be considered: (1) utilize and expand upon non-lethal and non-destructive sampling techniques, including eggshells and unhatched eggs, scute shell clippings, claw clippings, blood, and tail tips; (2) additional biometric data for reproductive female at each nest are required such as diet, size, age, and even telemetry if available; and (3) data on adjacent water and sediment chemistry (including Hg) as well as landscape features (% wetlands in watershed, proximity of contaminated sites and other potential sources).

Mi'kma'ki Acknowledgement Kwe'. It is a responsibility and an honour for us to work with mikjikjik, in Mi'kma'ki, the ancestral and unceded territory of the L'nu. We carefully collected eggshells from Common Snapping Turtle nests found near E'se'katik in Sipekne'katik with no harm to the turtles. The analytical work was carried out at Saint Mary's University in K'jipuktik, Sipekne'katik. We hope our data and the results will contribute to the continued recovery of all mikjikjik populations across Mi'kma'ki. Wela'lioq.

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